

(FILE 'HOME' ENTERED AT 15:58:18 ON 07 MAR 2001)

FILE 'CAPLUS, MEDLINE' ENTERED AT 15:58:27 ON 07 MAR 2001

L1 1397 S (FILTER (5A) HYBRIDIZ?)  
L2 980 DUPLICATE REMOVE L1 (417 DUPLICATES REMOVED)  
L3 0 S L2 AND ((INCREASE OR HIGH?) (P) TEMP? (P) WASH)  
L4 1 S L2 AND (REDUC? (P) BACKGROUND (P) SIGNAL)  
L5 0 S L2 AND ((INCREASE OR HIGH?) AND TEMP? AND WASH)  
L6 8 S (HYBRID? (P) (INCREASE OR HIGH?) (P) TEMP (P) WASH)  
L7 8 DUP REMOVE L6 (0 DUPLICATES REMOVED)  
L8 22 S HYBRID? AND (HIGH (5A) STRINGEN? (5A) WASH)  
L9 13 DUP REMOVE L8 (9 DUPLICATES REMOVED)

L7 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2001 ACS

AN 1999:520555 CAPLUS

DN 132:103373

TI Identification of oral Actinomyces species using DNA probes

AU Ximenez-Fyvie, L. A.; Haffajee, A. D.; Martin, L.; Tanner, A.; Macuch, P.;

Socransky, S. S.

CS Departments of Periodontology, Forsyth Dental Center, Boston, MA, USA

SO Oral Microbiol. Immunol. (1999), 14(4), 257-265

CODEN: OMIMEE; ISSN: 0902-0055

PB Munksgaard International Publishers Ltd.

DT Journal

LA English

AB Oral Actinomyces comprise a major segment of both the supra- and subgingival microbiota; however, little is known about the distribution

of individual species in different sites or clin. conditions. The purpose

of the present investigation was to develop DNA probes for suggested species and genotypes of oral Actinomyces. Whole genomic DNA probes to 12 human oral species and/or serotypes were labeled with digoxigenin and used to seek cross-reactions among the taxa using the checkerboard DNA-DNA **hybridization** assay. The Actinomyces formed three distinct groups: (1) Actinomyces georgiae, Actinomyces meyeri and Actinomyces odontolyticus serotypes I and II; (2) Actinomyces viscosus and Actinomyces

naeslundii serotypes I, II, III and WVA 963; and (3) Actinomyces gerencseriae and Actinomyces israelii. Cross-reactions among taxa were detected and minimized by increasing the **temp.** of the post-**hybridization high-stringency wash** to 80.degree.. Despite the elevation in **high stringency wash temp.**, cross-reactions among strains of the A. naeslundii A. viscosus group persisted. Probes for two of the three currently recognized genospecies in this group were prepd. by removing

the DNA in common between cross-reacting species using subtraction **hybridization** and polymerase chain reaction. Nine species and genospecies could be clearly sepd. by a combination of whole genomic and subtraction **hybridization** probes and by increasing the **high-stringency wash temp.** A total of 195 fresh isolates of Actinomyces were grouped in a blind study using DNA probes and sep. by SDS-PAGE protein profiles. Concordance between the

two methods was 97.3%. The probes and **hybridization** conditions were tested for their ability to detect the Actinomyces species and genospecies

in samples of supragingival and subgingival plaque from periodontitis subjects using checkerboard DNA-DNA **hybridization**. The probes detected the species in samples of supragingival and subgingival plaque. We concluded that whole genomic and subtraction **hybridization** DNA probes facilitate the detection and enumeration of species and genospecies of Actinomyces in plaque samples.

RE.CNT 52

RE

(1) Andersen, R; Infect Immun 1993, V61, P981 CAPLUS

(2) Bjourson, A; Appl Environ Microbiol 1992, V58, P2296 CAPLUS

(4) Bowden, G; J Dent Res 1993, V72, P1171 CAPLUS

(5) Caufield, P; J Clin Microbiol 1989, V27, P274 CAPLUS

\* L7 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2001 ACS  
 AN 1992:646606 CAPLUS  
 DN 117:246606  
 TI Use of high-temperature washing and reversible target capture to improve  
 the sensitivity of hybridization assays  
 IN Collins, Mark L.; Blomquist, Cecile; Lombardo, Massimo; Eldredge, John  
 PA Amoco Corp., USA  
 SO PCT Int. Appl., 44 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI	WO 9215708	A1	19920917	WO 1992-US1433	19920221
	W: JP				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE				
	EP 529070	A1	19930303	EP 1992-910601	19920221
	R: DE, FR, GB, IT				
	US 5702896	A	19971230	US 1996-598142	19960207
PRAI	US 1991-661917		19910227		
	WO 1992-US1433		19920221		
	US 1993-147906		19931103		

AB A method for improving the sensitivity of **hybridization** assays which reduces non-specific binding (NSB) and non-specific **hybridization** (NSH) is disclosed. The method includes a washing step utilizing tetra-alkylammonium salts at **high** temps., and release steps in which a probe-target complex is released from a solid support and recaptured. Use of both the washing and release steps results in substantial redn. in NSB and NSH without performing several rounds of release and recapture of the target nucleic acids. Using a single round of reversible target capture with **high-temp.** wash followed by release of the bound probe-target complex, picogram sensitivity was attained in detection of HIV RNA.

L9 ANSWER 3 OF 13 MEDLINE  
 AN 97443357 MEDLINE  
 DN 97443357  
 TI High-stringency subtraction for the identification of differentially regulated cDNA clones.  
 AU Scutt C P; Gilmartin P M  
 CS Centre for Plant Biochemistry and Biotechnology, University of Leeds, England, UK.  
 SO BIOTECHNIQUES, (1997 Sep) 23 (3) 468-70, 472, 474.  
 Journal code: AN3. ISSN: 0736-6205.  
 CY United States  
 DT Report; (TECHNICAL REPORT)  
 LA English  
 FS Priority Journals  
 EM 199801  
 EW 19980104  
 AB The technique of high-stringency subtraction described here facilitates subtractive **hybridizations** between directional cDNA libraries constructed in lambda ZAP II cloning vectors and represents an improvement on earlier methods for the subtraction of entire cDNA libraries. High-stringency subtraction is designed to eliminates the subtraction of differentially expressed cDNAs, which show similarity to constitutive sequences by the incorporation of a novel **high-stringency wash** step. This method also allows the size-selection of target cDNAs and incorporates an improved procedure for the synthesis of driver DNA used in subtractions.

L9 ANSWER 6 OF 13 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3  
AN 1991:649110 CAPLUS  
DN 115:249110

TI Molecular cloning of the secretory acid proteinase gene from *Candida albicans* and its use as a species-specific probe

AU Ganesan, K.; Banerjee, Anasua; Datta, Asis

CS Sch. Life Sci., Jawaharlal Nehru Univ., New Delhi, 110067, India

SO Infect. Immun. (1991), 59(9), 2972-7

CODEN: INFIBR; ISSN: 0019-9567

DT Journal

LA English

AB *C. albicans* secretes an acid proteinase when grown with a protein as a sole nitrogen source. The gene encoding this proteinase was isolated from

a genomic expression library of *C. albicans* constructed in  $\lambda$ gt10 by screening with antiproteinase antibodies. The affinity-purified antibodies used to verify the clones are monospecific; these do not cross-react with any other protein in the culture supernatants or crude exts. of *C. albicans* but strongly react with fusion proteins encoded by recombinant clones, revealing that these are true proteinase clones. Genomic Southern blot anal. shows that the proteinase gene is present at

a unique locus and that there is no other closely related gene in the *C. albicans* genome. The proteinase gene probe identified 2 transcripts on Northern blots (RNA blots), which are present at a much higher level in

C. *albicans* cells induced for proteinase secretion than in uninduced cells. The aspartyl proteinase gene reported earlier (T. J. Lott, et al. 1989)

is not that of secretory acid proteinase, since the N-terminal amino acid sequence of secretory acid proteinase does not correspond to the deduced amino acid sequence of the aspartyl proteinase gene. The secretory acid proteinase gene was used to probe Southern blots of genomic DNA of

several

medically important *Candida* species and *Saccharomyces cerevisiae*. Under **hybridization** and wash conditions of low stringency, *C. tropicalis* and *C. parapsilosis*, in addn. to *C. albicans* strains, gave specific signals, implying that *C. tropicalis* and *C. parapsilosis* have homologous secretory acid proteinase genes. However, under **wash** conditions of **high stringency**, signals were obtained only with *C. albicans* strains, suggesting that this gene can be used as a species-specific probe. A simple yeast colony **hybridization** technique is sufficient to distinguish *C. albicans* from other yeasts.

L9 ANSWER 7 OF 13 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 4  
AN 1991:509629 CAPLUS  
DN 115:109629  
TI A reliable method for Northern blot analysis using synthetic  
oligonucleotide probes  
AU Henderson, Gregory Stephen; Conary, J. T.; Davidson, J. M.; Stewart, S.  
J.; House, F. S.; McCurley, T. L.  
CS Sch. Med., Vanderbilt Univ., Nashville, TN, USA  
SO BioTechniques (1991), 10(2), 190-7  
CODEN: BTNQDO; ISSN: 0736-6205  
DT Journal  
LA English  
AB A method is developed for using short (30-42 base pair) synthetic  
oligonucleotide DNA probes in Northern blot assays. The method involves  
labeling the probes to **high** specific activity, very  
**stringent hybridization** and **wash** conditions,  
and the presence of several inhibitors of nonspecific binding in the  
**hybridization** buffer. This method was tested with several probes  
obtained from local and com. sources. The results with every probe used  
were high signal-to-noise ratios in an exposure time range of 30 min to 7

L9 ANSWER 8 OF 13 CAPLUS COPYRIGHT 2001 ACS  
 AN 1990:402970 CAPLUS  
 DN 113:2970  
 TI Nucleic acid **hybridization** probe for detecting fungi  
 IN Lemontt, Jeffrey F.  
 PA Integrated Genetics, Inc., USA  
 SO Eur. Pat. Appl., 21 pp.  
 CODEN: EPXXDW  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 335633	A2	19891004	EP 1989-303002	19890328
	EP 335633	A3	19910925		
	EP 335633	B1	19950503		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	JP 02150300	A2	19900608	JP 1989-76369	19890328
	AT 122103	E	19950515	AT 1989-303002	19890328
	US 5658726	A	19970819	US 1992-860295	19920327
PRAI	US 1988-173836		19880328		

AB A method is provided for constructing a cDNA (cDNA) probe for use in detecting in a sample, under conditions of predetd. stringency, a target organism belonging to a strain of fungi, but not detecting a ref. microorganism or any prokaryotic microorganism. The method comprises (1) detg. a nucleotide base region of small subunit rRNA (srRNA) of the target organism, the detd. sequence being in a srRNA region that is poorly conserved in eukaryotes and having no corresponding region in prokaryotes;

(2) comparing the sequence in (1) with the nucleotide base sequence in the corresponding srRNA region of the ref. microorganism, and selecting a subsequence within the detd. sequence as the probe site; and (3) synthesizing a cDNA complementary to the above useful probe site; the cDNA is the probe. Methods are also provided for constructing probes for detecting the target microorganism belonging to any of >1 predetd. strains of fungi or in the presence of >1 ref. microorganisms. Thus, total RNA isolated from *Candida albicans* was used as a template to obtain a cDNA sequence by the primer-extension method (Lane et al., 1985). The deduced RNA sequence of the *C. albicans* srRNA in the 642-805 region was compared to the corresponding regions from *Saccharomyces cerevisiae* (Nelles et al., 1984) by aligning the sequences. Inspection of the aligned sequences identified a 43-mer useful probe site, and the cDNA 43-mer to this site was prepd., purified, and 32P-labeled. The purified and labeled probe was tested for specificity by **hybridization** reactions with total RNA preps. from other strains of *C. albicans*, other *Candida* species, prokaryotic cells, and other eukaryotic cells. The probe detected strains of only 3 other *Candida* species besides the *C. albicans* controls: *C. tropicalis*, *C. parapsilosis*, and *C. guilliermondii* (weakly pos.). Following an initial low-stringency wash, all other strains were essentially neg. or only very slightly pos. with the probe. After a **high-stringency wash**, the same

hybridization pattern was obsd.; strains showing slightly pos.  
after low stringency showed as neg. under high stringency.



L9 ANSWER 10 OF 13 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 6  
AN 1989:472251 CAPLUS  
DN 111:72251  
TI Detection of proto-oncogenes in the genome of the amphibian *Xenopus laevis*  
AU Moreau, Jacques; Le Guellec, Rene; Leibovici, Michel; Couturier, Anne; Philippe, Michel; Mechali, Marcel  
CS Equipe Embryol. Mol., Inst. Jacques Monod, Paris, 75251, Fr.  
SO Oncogene (1989), 4(4), 443-9  
CODEN: ONCNES; ISSN: 0950-9232  
DT Journal  
LA English  
AB The *X. laevis* genome was probed by Southern blot anal. for the presence of

sequences homologous to mammalian or avian proto-oncogenes.  
**Hybridization** conditions were strictly defined with a known proto-oncogene to detect a pos. signal with DNA sequences having at least 60 to 64% homol. In such conditions 13 genes representing different oncogene families exhibited pos. **hybridizations** with specific DNA restriction fragments. Members of the protein kinase oncogene family were detected including *abl*, *erbB*, *fes*, *fms*, *ros*, *raf* and *mos*, *Ets*, *rel*, and the steroid hormone related receptor *erbA* also gave pos. signals with specific *Xenopus* DNA fragments. Proto-oncogenes *raf* and the *ras* family, *N-ras*, *H-ras* and *c-ral*, gave the strongest **hybridizations** and the signals remained pos. in **high stringency wash** conditions. This study confirms the relative conservation of these genes during evolution and opens the possibility of studying their role in one of the best characterized systems of embryonic development.